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### Methodological considerations

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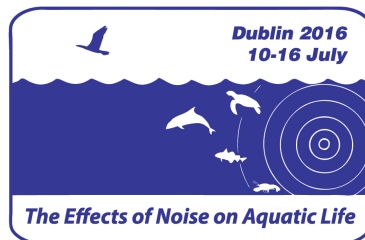
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## Fourth International Conference on the Effects of Noise on Aquatic Life

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## Underwater hearing in the great cormorant (*Phalacrocorax carbo sinensis*): Methodological considerations

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The underwater hearing threshold of a great cormorant (*Phalacrocorax carbo sinensis*) was measured at 2 kHz using psychophysical methods. Previous in-air and underwater testing suggests that cormorants have rather poor in-air hearing compared to other birds of similar size (Johansen, 2016). Preliminary data with a new underwater paradigm in a larger pool indicate that cormorants consistently respond to underwater sound and may therefore have special adaptations for underwater hearing.



## 1. INTRODUCTION

Over 800 species of birds rely on the marine environment for food or as their home. There is a vast range of adaptations in marine birds, from plovers barely sticking their head into the water or mud to the emperor penguin that can dive to depths of 500 m and for over 30 minutes (Meir et al., 2008).

Birds that dive to obtain their food may benefit from being able to hear underwater sounds. Sounds from prey as well as ambient underwater sound may help the bird while foraging as well as when orienting while diving. The in air hearing sensitivity of birds is well documented for many species (Dooling et al., 2002; Fay, 1988). However, data on the underwater hearing abilities of marine birds has previously been measured only in a few species (Crowell et al., 2015; Johansen et al., 2016; Larsen et al., 2016). Whereas marine mammals, such as seals and whales, have anatomical adaptations to improve their hearing abilities underwater (Thewissen and Nummela, 2008), there is currently very limited information on whether marine birds have any similar adaptations (Dooling and Blumenrath, 2016).

If marine birds are sensitive to underwater sounds, they may also be susceptible to effects of anthropogenic underwater noise. Underwater noise levels are increasing, and more evidence is collected that this affects a large range of species of aquatic animals (Ketten, 2008). When it comes to marine birds, however, we have no knowledge of the effect of human-induced sound sources (Crowell, 2016).

Previous studies on hearing in marine birds have been made by measuring the auditory brainstem responses (ABR) with birds under anesthesia (Larsen et al., 2016) or through behavioral studies using psychophysical methods with awake and alert animals (Johansen et al., 2016). ABR is the result of synchronized neural discharges from different regions of the brainstem as a response to acoustic stimuli (Hall, 2007). With this method data can be collected quickly and efficiently. However, thresholds derived with ABR are in general less sensitive than the ones derived using behavioral methods. Psychophysical studies, on the other hand, rely on training the bird to understand a paradigm, where it has to react correctly to a sound stimulus to obtain its reward (operant conditioning; Stebbins, 1970). Even though it takes much longer time to collect such data than using ABR, the benefit is that the derived thresholds not only incorporates signals generated by the brainstem, but also incorporates the decisions regarding whether or not a stimulus is detected in cortical areas of the brain. Due to these methodological differences there is a great need to standardize ABR and psychophysical methods so that they can be compared when determining underwater hearing thresholds from birds.

Here we study the underwater hearing sensitivity of a great cormorant, *Phalacrocorax carbo sinensis*, using 2 kHz tones as stimulus and a method of constant stimuli psychophysical paradigm. We compare the underwater threshold with recently derived in-air hearing thresholds for this species at the same frequency to evaluate how well adapted cormorants are for hearing under water (see Maxwell et al., this volume).

## 2. MATERIALS AND METHODS

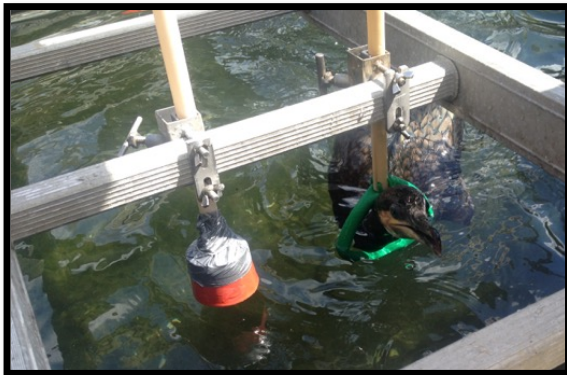
### A. Experimental subject

One 6-year-old, male great cormorant was tested. He weighed 2.6 kg, was wild born, and arrived at the Marine Biological Research Centre (University of Southern Denmark) in September, 2010. Since his arrival, he has received extensive training in classical and operant conditioning techniques for various psychophysics tasks, as well as a variety of husbandry and enrichment behaviors. He was housed in an outdoor aviary consisting of three rooms. The largest room, containing a large pool, also acted as the research pool.

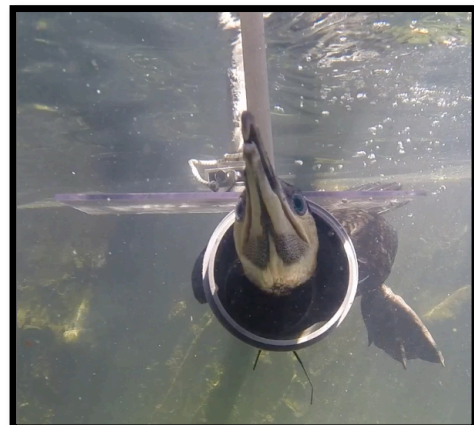
### B. Training

Positive reinforcement procedures were used with a GO/NO-GO testing paradigm. Initially, a set-up that was used for the in-air testing was placed at the surface (Figure 1). The set-up was then modified and approximated underwater to a depth of 30 cm for testing.

The subject swam voluntarily to an underwater station where he placed his head through the head station while his body rested against the plastic plate (Figure 2). For a GO-trial to be considered correct he left the station upon presentation of the tone-stimulus and touched the response target with his beak. This was marked by a conditioned reinforcer, a dog whistle, followed by an unconditioned reinforcer, a fish reward given by the trainer. If he did not respond within the 4 s response time, his response was considered incorrect and no reinforcement was given. For a correct NOGO-trial, he was required to stay in the station until the end of the trial (4 seconds). An underwater conditioned reinforcer (0.5 s broadband buzz) marked the end of the trial and a fish reward was given.



*Figure 1. Initial training at surface of water with in-air setup*



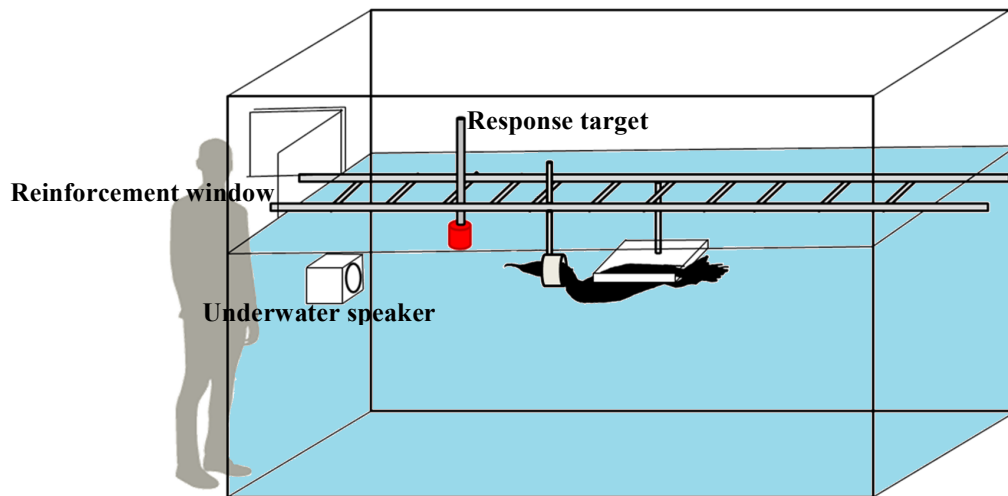
*Figure 2. Underwater station position*

### C. Apparatus

The experiment was conducted in a rectangular pool (4.0 m x 2.5 m) with a water depth of 1.5 m. The speaker was mounted underwater half-way along the longest pool wall at a depth of 30 cm and aligned with the station with a distance of 60 cm between them. The underwater head station consisted of a 20 cm long polyvinyl chloride (PVC) tube to minimize head movement and

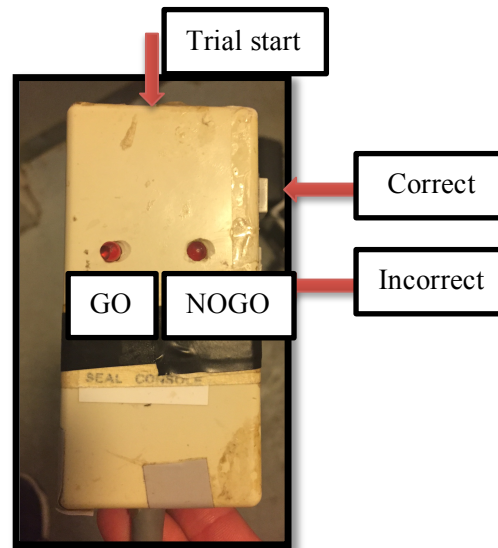
a clear plastic plate that assisted in keeping his body motionless during trials. The GO-response target was located at the water's surface to the left of the underwater station.

Each trial was started and the trainer gave reinforcement from the observation window at the edge of the pool (Figure 3).



*Figure 3. Experimental setup.*

To ensure impartiality, the trainer must not know whether the trial is a signal-present or a signal-absent trial until after the subject has responded; therefore a double blind procedure was established to avoid any experimenter bias. A small handheld console was controlled by the trainer and functioned as a double blind (Figure 4). It contained a trial start button, a correct response button, which also triggered the underwater conditioned reinforcer, and an incorrect response button. Once the trainer hit the trial start button a preprogrammed randomizing sequence initiated either a stimulus-present (GO) or a stimulus-absent (NOGO) trial. A lamp on the console indicated trial type upon completion of the trial. Both trial types were initiated in the same way with the only difference being that no stimulus was presented in NOGO trials. For GO trials, the stimulus was presented 1-2 s after the start of the trial. It was not possible for the trainer to hear the stimulus tone during testing. Each trial had a duration of 4 s. The total number of trials, the number of correct responses, incorrect responses, false alarms, misses, as well as the relative intensity of the sound stimulus for each stimulus-present trial was registered and conveyed to a laptop at the completion of each session.



**Figure 4. Trial console.** The trial start button initiated either a signal-present or a signal-absent trial. At the completion of the trial, either the GO or NOGO lamp turned on, indicating to the trainer which trial type had been presented to the bird.

## D. Stimulus

The underwater stimulus was a 0.5 s pure tone at 2 kHz, that included a 100 ms ramp-up and 100 ms ramp-down to avoid spectral smearing. The stimulus and the sound field were measured once per week using a pistonphone calibrated ( $\pm 1$  dB) Reson TC4032 hydrophone connected to an Olympus LS-10 digital recorder. The intensity of the generated WAV file was measured using a custom-built Matlab program (rms of the duration of the signal defined by the 95 % cumulative energy function). The paradigm was steered through a LabView program on a laptop connected with USB to a DAQPAD (National Instruments USB-9162). The output of the DAQPAD was connected to a Basetech AP-2100 amplifier and to an underwater loudspeaker (University Sounds UW-30 or a Lubell model LL9162T). The ambient noise levels were recorded once per day using the same system. All recordings were done in the testing tank with the subject not present.

## E. Testing parameters

Using the method of constant stimuli, four stimulus levels with 6 dB increments had a measured received level (i.e., stimulus strength at the position of the bird's head) of 48, 54, 60, 66 dB re 1  $\mu$ Pa rms. Each level was tested at least two times per session, using a 50/50 GO/NOGO ratio. To avoid any predictability in trial type, 12 separate pseudorandom programs with a set ratio of stimulus-present and stimulus absent trials were created (Gellerman, 1933). There were never more than three trials in a row of a given type (GO or NOGO) to avoid the possibility of the subject predicting the subsequent trial type based on previous trials.

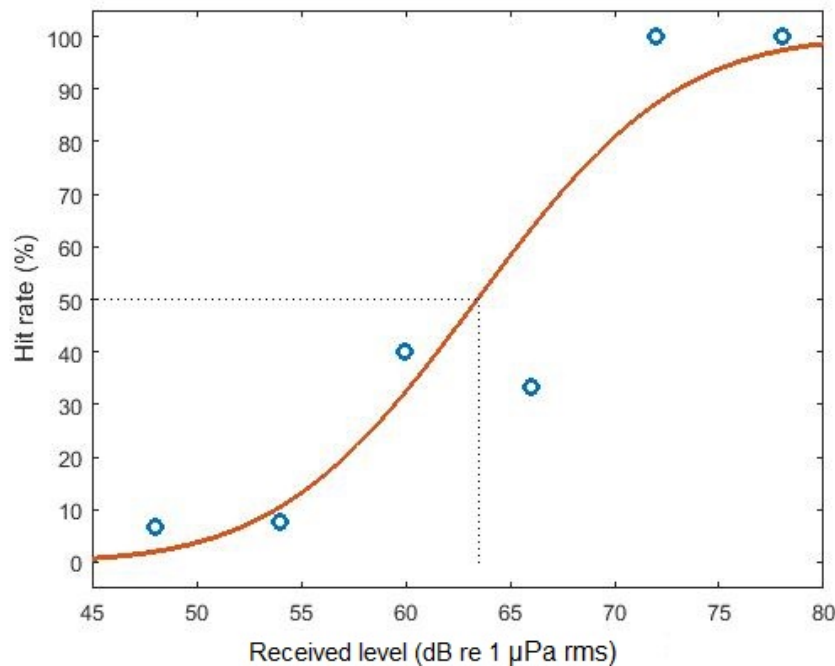
For all correct responses (correct detections and correct rejections) the bird received a fish reward of 8 g. For incorrect responses, false-alarms or misses, no reward was given. One to two data sessions were conducted per day, 5 days a week. Each session consisted of at least 20 trials,



including warm-up and cool down trials. For the warm-up and cool down trials, the signal stimulus level was well above threshold. The subject's daily diet was established to maintain a healthy body mass and was not constrained for experimental purposes.

### 3. RESULTS

The underwater hearing threshold for 2 kHz tones was determined to be 64 dB re 1  $\mu$ Pa rms from the 50 % correct detection level of the psychometric (Probit) function fitted to the data (Figure 5). The false alarm rate and hit rate were 8.5 % and 59 %, respectively.



**Figure 5. Underwater hearing threshold at 2 kHz for the great cormorant**

### 4. DISCUSSION

The behavioral underwater hearing threshold obtained here at 2 kHz is 29 dB lower than a previously measured ABR underwater threshold from cormorants at the same frequency (Larsen et al., 2016). It is common that behavioral paradigms result in substantially lower hearing thresholds than ABR paradigms (Brittan-Powell et al., 2002).

The hearing threshold is also some 11-18 dB lower than previous behavioral measurements made on the same species (Johansen et al., 2016). The interpretation of the previous estimates are however difficult, as there was considerable spreading in the data. This is probably due to lack of behavioral control of the bird and larger variation in the background noise levels. In addition, the previous measurements were made in a very small tank, with the bird having its body in air and only submerging its head during the trials. This may have affected both the sound field and the hearing system of the bird as compared to the current setup with the animal completely submerged in a larger tank. The current behavioral paradigm seems robust and is also seemingly generating reduced variation between trials as compared to the previous study by Johansen et al. (2016).



Recent psychophysical measurements of the same individual (see Maxwell et al., this volume) resulted in an in-air hearing threshold of 13 dB re 20 $\mu$ Pa rms at 2 kHz. When comparing in-air and underwater decibels it is important to note that 62 dB should be added to the in-air numbers to produce an equivalent underwater sound intensity (see e.g., Larsen and Wahlberg, in press). Thus, if the cormorant had a similar hearing sensitivity in air as in water, we would have expected to measure an underwater hearing threshold of 13+62=75 dB re 1  $\mu$ Pa rms. As we measured a threshold that is 11 dB lower than 75 dB, this may suggest that great cormorants have special adaptations for underwater hearing.

Cormorants are known to live in colonies with up to several thousand individuals and to forage socially (Grémillet et al., 1998). The ability to hear as well underwater as in air can have numerous advantages. With birds in air, hearing serves three purposes: 1) communication, 2) navigation and 3) prey detection (Sibley, 2001). It can be assumed that these same purposes would apply to submerged marine birds as well; even more so for birds that dive to the deeper depths or hunt during dawn or dusk where light may be a limiting factor.

Further studies of underwater hearing abilities of marine birds, as well as data from more than one individual, are needed to understand the full range of adaptations and assess their sensitivity for anthropogenic noise disturbances. Obtaining critical ratio and directional hearing data would be particularly interesting to determine how anthropogenic noise may affect marine birds in their amphibious habitats.

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